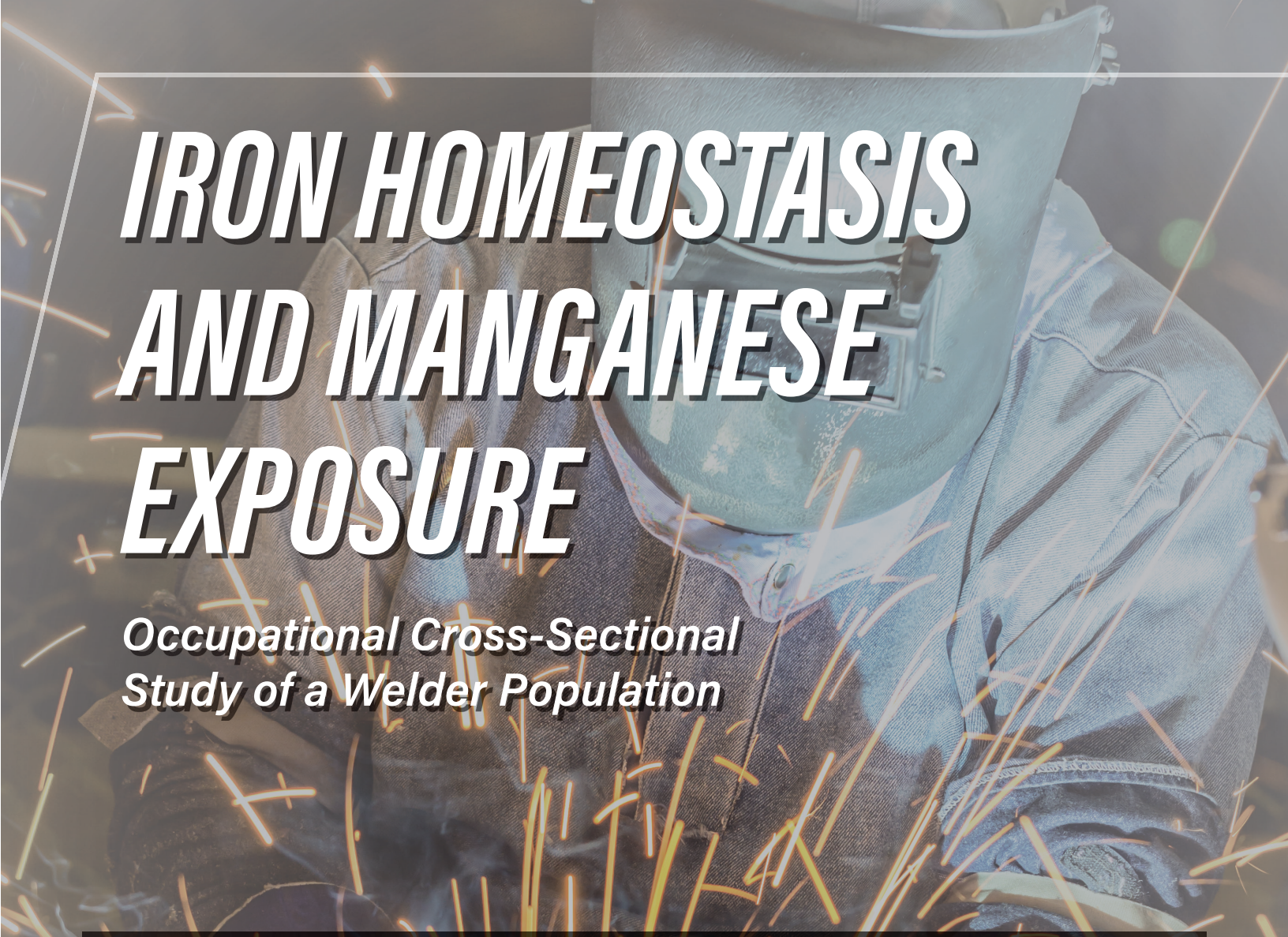

IRON HOMEOSTASIS AND MANGANESE EXPOSURE

Occupational Cross-Sectional Study of a Welder Population

Abstract

Manganese exposure is a serious occupational health hazard for many welders, smelters, and miners. Manganese exposure may result in Parkinson-like symptoms. It has been hypothesized that altered iron concentrations in the brain could also lead to neurological symptoms similar to what appears in those with chronic exposer to manganese. Therefore, the goal of this project is to explore the relationship between manganese and hepcidin as an indicator of altered iron homeostasis. Population demographics were collected from semi-trailer factory workers in a cross-sectional study via a questionnaire. Demographic information includes age, race, and weight. Toenail clippings and questionnaires were collected on the same day; blood and air concentrations were sampled on a different day within a short time period. Statistical analyses include descriptive statistics and regression analyses. Multiple linear regressions were run that utilized hepcidin and transferrin as dependent variables, with air, toenail, and blood manganese and welder status as independent variables. Mean air concentrations for the metals were below recommended exposure limits. Manganese toenail concentrations were 5.23 μg/g (standard deviation [SD] = 2.53 μg/g) while iron toenail concentrations were 183.93 μg/g (SD = 124.06 μg/g). Results showed a statistically significant association between natural log (ln)(hepcidin) and welding status ($p = 0.042$); no statistically significant relationship was found between ln(hepcidin) with air manganese ($p = 0.313$) or toenail manganese ($p = 0.672$). There was no association found between transferrin and air manganese, toenail manganese, or welder status. Continued research is needed to investigate these relationships further.

Keywords

manganese, iron homeostasis, hepcidin, occupational health, epidemiology.

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INTRODUCTION

Workers in industries such as welding, smelting, and mining work in conditions that result in elevated exposure to several metals, including manganese. Manganese is classified as a trace metal and commonly serves as an enzyme cofactor in many metabolic functions, including immune response, blood clotting, and bone production (US Department of Health and Human Services, 2019). However, exposure to high concentrations of manganese can lead to neurological deficits such as tremors, slowness of movement, irritability, loss of concentration, and lapse in memory (Williams Todd, Roney et al., 2012). All of these symptoms are found in manganism, a condition with Parkinson's disease–like symptoms that may occur in those with very high manganese exposure (Andruska and Racette, 2015; Centers for Disease Control and Prevention, 2019). Chronic exposure to somewhat lower concentrations of manganese may lead to neurological damage that includes neuronal inflammation, decreased cognitive function, and altered behavior (Williams et al., 2012). There is concern that welders may be exposed to sufficient manganese to experience these types of health impacts (Williams et al., 2012).

While there is a clear indication that manganese causes neurotoxicity, the exact mechanism of injury is still unclear. One hypothesis is that manganese interferes with the function of proteins involved in iron homeostasis (Bjørklund, Dadar, Peana, Rahaman, & Aaseth, 2020). Manganese and iron share similar chemical characteristics, allowing manganese to interact with proteins that would traditionally use iron as a cofactor (Zheng, Fu, Dydak, & Cowan, 2011). Iron itself has been shown to lead to an increased risk of Alzheimer's and Parkinson's disease (Wang et al., 2016). This occurs because of iron's role in redox reactions, leading to increased levels of oxidative stress and protein aggregation in cases of iron accumulation in the brain (Salvador, Uranga, & Giusto, 2010). Thus, due to the close nature of iron and manganese, it is possible that manganese may affect neurological function via its ability to alter iron homeostasis.

Iron serves many diverse functions but primarily assists in the transport and storage of oxygen around the body when bound to hemoglobin and myoglobin (King, 2014). Because of its vital function, iron is tightly regulated

directly at its location of absorption, with multiple homeostatic mechanisms regulating the process (Himmelfarb, 2007; Abbaspour, Hurrell, & Kelishadi, 2014). Hepcidin, a peptide hormone, is considered the main iron-regulatory mechanism that inhibits the transport of iron into the bloodstream. This occurs when hepcidin binds to ferroportin, a protein that sits on top of cells that act as absorption sites. Once bound, ferroportin retracts from the surface cells and degrades, blocking iron from entering into plasma cells, the primary cellular mechanism of delivery of the metal throughout the body (Abbaspour et al., 2014) Although hepcidin acts on ferroportin, it can only react to fluctuating iron levels within the body if molecular iron is bound to transferrin, another plasma glycoprotein (Nemeth & Ganz, 2009). The response from hepcidin is proportional to the amount of iron that is attached to transferrin that is in the plasma (Collins, Wessling-Resnick, & Knutson, 2008).

Iron homeostasis is a complex process with ample opportunity for the potential influence of other factors not directly involved in the chief iron-homeostatic pathway. Manganese is one of these potential influencers and has been suggested in several studies to have an effect on iron storage via an increase in iron-hormone levels (Seo & Wessling-Resnick, 2015; Bjørklund et al., 2017; Harischandra et al., 2019). The goal of this project is to explore the relationship between manganese and hepcidin as an indicator of altered iron homeostasis. While transferrin and hepcidin's role in iron homeostasis is well established in the scientific community, the role the hormone plays in manganese metabolism is still debated. There is currently limited data in this area, particularly in humans; therefore, this analysis is important, as it will provide additional results from a key human population, those with occupational manganese exposure.

METHODS

This analysis is based within a larger longitudinal study of workers at a semi-trailer factory in Indiana, described in detail previously (Ward, Edmondson, Nour, Snyder, Rosenthal, & Dydak, 2017; Ma et al., 2018). The current analysis is a cross-sectional analysis of the subset of participants who provided blood samples. The study was approved by the Institutional Review Board of Purdue University, and all participants provided written informed consent prior to participation.

Data used in this analyses were collected from 39 participants: 21 welders (with occupational manganese exposure) and 18 nonwelder shift workers (without occupational manganese exposure) during 2015–2016. Data for several measurements were missing for some of these workers; specifically, 33 air samples, 19 toenail samples, and 25 blood samples were analyzed for manganese concentrations.

Data were collected via questionnaires (demographics, work history), biomarkers (blood, toenails), and environmental sampling (personal air samples). The questionnaire was used to collect demographic data consisting of age, sex, race, smoking habits, alcohol use, and weight. A detailed work history was taken; this included current employment details, previous employment, and off-the-job activities such as hobbies or side jobs that involve welding. As part of the work history, department and welding status were recorded. A food-frequency questionnaire was also given to estimate manganese that the participants consumed.

Blood samples were collected by a professional phlebotomist around an hour after each worker's shift ended. Vacutainers specifically designed for trace metal analysis were used to collect samples tested for manganese. The serum samples were allowed to sit for 30 minutes so separation could take place and then centrifuged; whole blood and serum samples were kept in a −80C freezer until they were analyzed for metal and biological sample concentrations at professional laboratories. Blood samples were analyzed for manganese, hepcidin, transferrin, and C-reactive protein.

As described previously (Ward et al., 2017), toenail samples consisted of clippings taken from all toes. External contamination was removed by sonication with a 1% Triton X-100 surfactant solution for 20 minutes and then rinsed with deionized water. The toenails were then dried, weighed, and dissolved using microwave nitric acid digestion. Samples were analyzed by inductively coupled plasma-mass spectrometry by Purdue University's Campus-wide Mass Spectrometry Center.

Methods for the collection of personal air samples have been described previously (Ward et al., 2017). Briefly, a SKC 25mm aluminum cyclone with an aerodynamic diameter cut point of 4.0 μm, in line with SKC Airchek 52 personal sampling pumps that absorbed 2.5 liters of air per minute, were used to sample the factory air. The cyclone contained a 25mm mixed cellulose ester filter that had a pore size of 0.8 μm weighted matched to 50 μg, which was placed inside the helmet of each welder. The cyclone was then placed on the worker's shoulders. The filters were stored in temperature- and humidity-controlled rooms before analysis and were then weighed and recorded. The NIOSH method 7300 was used with small variations. Samples were analyzed by plasma-mass spectrometry by Purdue University's Campus-wide Mass Spectrometry Center. A cumulative estimate of air manganese exposure for each worker in the study was estimated using a weighted equation that incorporated data from the work history, dietary history, and personal air–monitoring results.

The demographic and concentration data were analyzed for the 39 subjects taken from the initial data set within Excel and SPSS. Mean and range data were found for age and weight. Race, tobacco use, and alcohol use are presented as the number of participants who fell into each category and the percentage of that number for the total population. Tobacco and alcohol use were broken into separate categories based on amount of use such as nonsmoker, former smoker, and smoker; alcohol use was grouped by use of zero to three times per week and three or more times per week. The mean and SD were then found for blood manganese, air manganese, air iron, toenail manganese, and toenail iron.

The preliminary statistical analysis was run on the data in the form of unadjusted linear regressions. Variables that were not normally distributed (manganese biomarkers, C-reactive protein) were natural-log transformed prior to inclusion in models. Each regression compared one independent variable to one other variable. For this analysis, the main outcome variables were hepcidin and transferrin, and the main predictor variables were air manganese, toenail manganese, blood manganese, and welder/nonwelder status.

Each multiple linear regression covariate was chosen to account for potential confounding of the relationship between manganese concentrations and iron-homeostatic hormones. Metabolic activity of metals can change based on age and weight; the more people weigh and the older they are, the slower their metabolism and the higher tendency they have to retain heavy metals (Hensrud, 2019; Chang, Shen, Zhang, Song, & Jiang, 2018). C-reactive protein is made by the liver and was used to evaluate for inflammation in the body. This was included to assess whether the inflammation was a predictor of hepcidin or transferrin.

Based on results from unadjusted models (data not shown), multiple linear regression models were constructed to determine the relationship between hepcidin or transferrin as outcome variables with either air manganese, toenail manganese, or welder/nonwelder status as the exposure variable. Blood manganese was not included as an exposure variable due to the fact that blood manganese was not significantly associated with either hepcidin ($p = 0.648$) or transferrin ($p = 0.887$) in unadjusted regression models. This is in contrast to air and toenail manganese, which indicated a potential relationship from the initial unadjusted linear regressions with hepcidin (air manganese $p = 0.090$, toe manganese $p = 0.131$) and transferrin (air manganese $p = 0.356$, toe manganese $p = 0.286$).

RESULTS AND DISCUSSION

Population demographics indicated a wide range in backgrounds and health indicators (Table 1). The average age of subjects was 43.9 years (SD = 10.2 years). The vast majority of participants were male (n = 35, 89.74%); as there were too few females to be able to estimate any data for this group, we did not include sex as a variable in models. A majority of participants were white; nonwhite participants included those identifying as African American, north African, and Hispanic. The mean weight was 220.2 pounds (SD = 37.1 pounds). The majority of participants reported being nonsmokers (69.2%) and only consuming alcohol 0–3 times per week (89.7%).

Table 2 presents the average concentrations of each metal biomarker. Average air manganese concentrations (0.06 mg/m^3) were recorded to be just above the American Conference of Governmental Industrial Hygienists' recommended exposure limit of 0.02 mg/m^3 during an

eight-hour time-weighted average (3M, 2016). Average blood manganese concentrations (10.82 ug/L) were within the expected range of 4.7–18.3 ug/L (manganese, blood). Toenail manganese and iron levels were similar to those found in several other studies evaluating occupational exposure to manganese at 5.23 ug/g and 183.93 ug/g, respectively (Bakri, Hariri, Ma'arop, & Hussin, 2017).

TABLE 1. Population demographics (n = 39).

Variable	Measure	Value(s)
Age, years	Range	22.27–61.69
	Mean (SD)	43.91 (10.24)
Race	White, n (%)	30 (76.92)
	Nonwhite, n (%)	9 (23.08)
Weight, pounds	Range	153–310
	Mean (SD)	220.20 (37.19)
Smoking status	Nonsmoker, n (%)	27 (69.23)
	Former smoker, n (%)	7 (17.95)
	Smoker, n (%)	5 (12.82)
Alcohol use	0–3 times/week, n (%)	35 (89.74)
	> 3 times/week, n (%)	4 (10.26)

SD = standard deviation.

TABLE 2. Manganese and iron concentrations.

Sample	N	Mean (SD)
Blood Mn (µg/L)	25	10.82 (5.81)
Air Mn (mg/m^3)	33	0.06 (0.007)
Toenail Mn (ug/g)	19	5.23 (2.53)
Air Fe (mg/m^3)	30	0.62 (0.83)
Toenail Fe (ug/g)	17	183.93 (124.06)

SD = standard deviation, Mn = manganese, Fe = iron.

Results for multiple regression models predicting hepcidin are shown in Table 3. Hepcidin was included as a natural-log transformation in these models. These models also adjusted for age, weight, and C-reactive protein (air and toenail manganese only). There was no significant association of ln(hepcidin) with air manganese ($p = 0.313$) or toenail manganese ($p = 0.672$). However, we did observe a statistically significant association of ln(hepcidin) with welding status: specifically, welders had significantly higher hepcidin levels compared to nonwelders ($p = 0.042$).

Results for multiple regression models predicting transferring are shown in Table 4. Similar to the models for hepcidin, transferrin was included as an ln-transformed variable, and the regression models included the same covariates as in Table 3. No association was observed between air manganese ($p = 0.530$), toenail manganese ($p = 0.619$), and welder status ($p = 0.838$) with transferrin.

C-reactive protein was not a significant predictor in the adjusted models for either hepcidin or transferrin. These data suggest that factors other than inflammation may be important in predicting hepcidin or transferrin in the presence of manganese.

Some prior research also explored whether manganese was associated with hepcidin or transferrin. Many of the studies investigated the role that iron hormones had in manganese metabolism, whereas this study attempted to find an association between manganese exposure and iron

TABLE 3. Multiple linear regression results for prediction of hepcidin.

Variable	β Coefficient (95% Confidence Interval)		
	Model 1 (Air Mn)	Model 2 (Toenail Mn)	Model 3 (Welder Status)
N	19	16	21
Air Mn, mg/m^3	0.064 (−0.067, 0.194)	—	—
Toenail Mn, ug/g	—	0.026 (−0.106, 0.158)	—
Welder (vs. nonwelder)	—	—	0.561 (0.024, 1.099)
Age, year	0.009 (−0.019, 0.037)	0.013 (−0.017, 0.043)	0.002 (−0.026, 0.029)
Weight, lbs.	0.002 (−0.005, 0.009)	0.001 (−0.006, 0.009)	0.001 (−0.006, 0.007)
ln(CRP), mg/L	0.085 (−0.144, 0.310)	0.061 (−0.017, 0.290)	—

Each model is adjusted for all variables in the table. Hepcidin is included in models as ln(hepcidin). Mn = manganese, CRP = C-reactive protein, ln = natural log.

TABLE 4. Multiple linear regression results for prediction of transferrin.

Variable	β Coefficient (95% Confidence Interval)		
	Model 1 (Air Mn)	Model 2 (Toenail Mn)	Model 3 (Welder Status)
N	13	11	22
Air Mn, mg/m^3	−2.968 (−13.404, 7.468)	—	—
Toenail Mn, ug/g	—	−2.240 (−12.699, 8.219)	—
Welder (vs. nonwelder)	—	—	−2.825 (−31.515, 25.864)
Age, year	−0.216 (−2.559, 2.127)	0.019 (−2.609, 2.648)	−0.148 (−1.722, 1.426)
Weight, lbs.	−0.043 (−0.591, 0.506)	−0.007 (−0.623, 0.609)	0.095 (−0.303, 0.494)
ln(CRP), mg/L	3.623 (−16.604, 23.849)	−0.525 (−25.906, 24.856)	—

Each model is adjusted for all variables in the table. Transferrin is included in models as ln(transferrin). Mn = manganese, CRP = C-reactive protein, ln=natural log.

homeostasis, limiting our ability to make direct comparisons (Bartnikas, 2012; Casjens et al., 2014; Bjørklund et al., 2020). One study suggested that transferrin plays an indirect role on hepcidin expression and/or iron metabolism when there are tissue manganese abnormalities observed (Herrera, Pettiglio, & Bartnikas, 2014). Another study indicated that hepcidin did not affect manganese metabolism in a mouse model (Jin et al., 2019).

This study was limited by its small sample size. If this study is ever revisited, an increased study population could help to better determine if manganese concentrations can be used as a predictor to iron-hormone levels present in the body. As this was a cross-sectional study, we cannot completely establish a temporal relationship; thus, a longitudinal study where manganese samples could be compared with changes in hepcidin or transferrin over time would also be helpful to further describe these associations.

This study also has several strengths that included the use of data from a human population when compared to similar studies that utilized animal and microbial populations. The amount of detailed information gathered on manganese exposure was another strength that allowed for the investigation of unique relationships that otherwise would have been difficult to evaluate without those measurements.

CONCLUSIONS

The goal of this cross-sectional study was to evaluate the association between manganese and the iron-homeostatic mechanisms of hepcidin and transferrin as an indicator of altered iron homeostasis. No association between air manganese or toenail manganese with measures of iron homeostasis were observed; however, welders were observed to have a statistically significant higher concentration of hepcidin compare to nonwelders. More research is recommended to further evaluate these relationships.

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